

Sulfur Amino Acid Activity of D- and L-Homocysteine for Chicks¹ (40008)JEANNINE M. HARTER AND DAVID H. BAKER²*Department of Animal Science, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801*

Among the indispensable amino acids, methionine is unique because both of its isomers have equal nutritive value when added to a diet at levels near the animal's dietary requirement (1). This raises speculation concerning the biopotency of the D and L isomers of homocysteine, since homocysteine is a normal intermediate in the conversion of methionine to cysteine. Definitive information is lacking concerning efficacy of the isomers of homocysteine as sources of methionine per se or cysteine per se.

In virtually all nutritional research with homocysteine, the DL-racemic mixture has been used. Moreover, cysteine status of the animal has not been clearly defined. The resulting data have therefore been difficult to interpret. Conflicting reports appear in the literature (2-9) regarding the efficacy of homocysteine as a source of sulfur amino acids (SAA). The earliest work by du Vigneaud *et al.* (2) indicated stimulation of growth in rats when DL-homocysteine was added to a cystine-deficient diet. They suggested, however, that both isomers were not equal in biopotency. In subsequent work, Dyer and du Vigneaud (4) observed that growth of rats fed D-homocysteine in a cystine-deficient diet was equal to that of rats fed the L isomer, although both isomers were inferior to L-cysteine.

The transmethylation of homocysteine to methionine (Fig. 1) has become a generally accepted concept based upon early growth data with rats and chicks (5-7). The original research was done without the aid of a carefully defined purified diet and, as such, nutrient imbalances were common. Rose and Rice (5) observed responses in rats to DL-homocysteine when added to a SAA-free diet, but growth was

still inferior to that of rats fed DL-methionine. du Vigneaud and co-workers (6), feeding a purified diet similar to that of Rose and Rice, observed that growth of rats fed DL-homocysteine equaled that of rats fed DL-methionine. It should be noted, however, that rats fed DL-methionine gained only 1 g/day, a growth rate of questionable magnitude for evaluating transmethylation activity.

The purpose of our assays was to evaluate the D and L isomers of homocysteine³ as sources of either methionine or cysteine for chicks fed a purified L-amino acid diet that allowed strict control of methionine and cysteine status.

Materials and methods. Male chicks resulting from the cross of New Hampshire males and Columbian females were used in all assays. Care of chicks prior to the start of each assay and procedures of experimental allotment have been described (10). The basal diet (Table I) was devoid of sulfur amino acids, and all additions were made at the expense of cornstarch. Feed and water were offered *ad libitum*. Growth and feed efficiency were measured. Data were analyzed by analysis of variance techniques, and pooled standard errors were calculated for each response parameter.

Assay 1 was designed to determine the efficacy of the D and L isomers of homocysteine as sources of methionine or cysteine. A cystine-deficient diet series and a methionine-deficient diet series were fortified with either D- or L-homocysteine, and performance was compared with an equimo-

³ L- and D-homocysteine were provided as homocysteinethiolactone hydrochloride (De Gussa, Inc.), a compound equal to homocysteine in biological activity. Male chicks in our laboratory fed SAA-deficient diets to which were added isosulfurous quantities of DL-homocysteine or DL-homocysteine-thiolactone·HCl from Day 8 to Day 20 posthatching gained 129.5 and 129.6 g, respectively.

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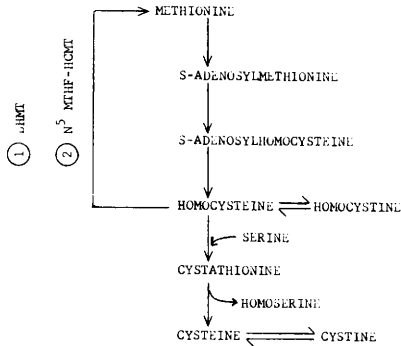


FIG. 1. Scheme of homocysteine interconversion. ⓐ BHMT, Betaine-homocysteine methyltransferase; ⓑ N^5 MTHF-HCMT, N^5 -methyltetrahydrofolate-homocysteine methyltransferase.

lar level of L-cystine or L-methionine, respectively. In the cysteine series, sufficient methionine (0.30%) was present to meet the chick's physiological requirement and, in the methionine series, sufficient cystine (0.25%) was provided to cover the physiological requirement for cystine (11).

Assay 2 was designed to ascertain if performance of chicks fed graded levels of L-homocysteine as a source of methionine could be made equal to that of chicks fed L-methionine itself. L-Cystine was included in all diets at 0.35% to ensure that it would not be growth limiting. Treatments (Table III) consisted of graded levels of L-methionine to establish a standard curve and graded levels of L-homocysteine to generate a dose-response curve that could be related to the L-methionine standard curve. The slope-ratio approach (10, 11) was used to determine the efficacy of L-homocysteine as a source of methionine.

Comparative growth of chicks fed L-homocysteine as a source of methionine in diets containing two levels of choline was evaluated in assay 3. All diets were made adequate in L-cystine, and isosulfurous levels of L-methionine and L-homocysteine were fed. Choline chloride was included in the diet at either 0.20 or 0.50%.

Assay 4 evaluated homocysteine in diets with and without dietary cystine. Work by various researchers (2-9), which had shown homocysteine to be a good source of methionine, was done with diets that were limiting in both cysteine and methionine.

Thus, homocysteine was serving as a source of total sulfur amino acids (TSAA) in these studies rather than as a source of methionine alone. Assay 4 was therefore designed to determine if methionine and cysteine must both be limiting in order for homocysteine to be utilized efficiently. DL-Homocysteine⁴ was used in this assay due to exhaustion of the supply of L-homocysteinethiolactone hydrochloride.

Results and discussion. Chicks fed L-homocysteine as a source of cysteine gained as fast and as efficiently as those fed L-cystine (Table II). Relative gain was reduced to 68%, however, for chicks fed D-homocysteine. Both L- and D-homocysteine were poorly utilized as sources of methionine in diets where the cysteine requirement had been met by provision of 0.25% L-cystine. Relative gain for the 8-day assay for chicks fed L-homocysteine was 28% that of chicks fed L-methionine, and only 7% when D-homocysteine was fed. These results indicate that the chick fed a cysteine-adequate diet cannot efficiently convert either L- or D-homocysteine to methionine. In contrast, chicks fed methionine-adequate diets can convert homocysteine to cysteine much more efficiently. To our knowledge, this has been the first work to quantify the transmethylation conversion of D- and L-homocysteine to methionine under conditions of cysteine adequacy. However, homocysteine utilization has been studied by Dyer and du Vigneaud (4) and du Vigneaud *et al.* (2) in rats fed diets deficient in cysteine.

Results of assay 2 are shown in Table III. Chicks responded linearly to graded levels of L-methionine resulting in a standard curve with a slope of 36.44 g of gain/mole of L-methionine intake as shown in Fig. 2. Chicks fed graded levels of L-homocysteine responded in a manner best described by a broken-line least-squares model as shown in Fig. 2. Chicks with intakes of L-homocysteine up to 0.765 mmole gained 28.30 g/mmole of intake. Chicks with intakes greater than 0.765 mmole of L-homocysteine gained only 5.60

⁴ DL-Homocysteine supplied by De Gussa, Inc., West Germany.

TABLE I. COMPOSITION OF BASAL DIET

Ingredient	(%)	Amino acid mixture	(g/19.78 g)
Cornstarch	to 100	L-Arginine · HCl	1.15
Amino acid mixture	19.78	L-Histidine · HCl · H ₂ O	0.45
Corn oil	15.00	L-Lysine · HCl	1.14
Salt mixture ^a	5.37	L-Tyrosine	0.45
Cellulose ^b	3.00	L-Tryptophan	0.15
NaHCO ₃	1.00	L-Phenylalanine	0.50
Choline chloride	0.20	L-Methionine	—
Vitamins ^a	0.20	L-Cystine	—
α -Tocopherol acetate (20 mg/kg)	+	L-Threonine	0.65
Ethoxyquin (125 mg/kg)	+	L-Leucine	1.00
		L-Isoleucine	0.60
		L-Valine	0.69
		Glycine	0.60
		L-Proline	0.40
		L-Glutamic acid	12.00
		Total	19.78

^a Katz and Baker (10).

^b Solka Floc, Brown Company, Chicago, Ill.

TABLE II. PERFORMANCE OF CHICKS FED D- AND L-HOMOCYSTEINE AS A SOURCE OF EITHER METHIONINE OR CYSTINE (ASSAY 1)^a

Diet	Level of SAA (%) ^b				Gain (g)	Relative gain (%)	Gain/feed
	L-M	L-C	L-HC	D-HC			
1	0.30	—	—	—	39.4	35	0.40
2	0.30	0.25	—	—	113.9	100	0.72
3	0.30	—	0.28 ^c	—	110.0	97	0.71
4	0.30	—	—	0.28 ^c	77.4	68	0.59
5	—	0.25	0.27 ^d	—	32.3	28	0.44
6	—	0.25	—	0.27 ^d	7.9	7	0.16
7	0.30	0.25	0.28	—	103.8	91	0.71
8	0.30	0.25	—	0.28	101.2	89	0.72
Pooled SE					2.4	3	0.01

^a Average of triplicate groups of seven male chicks for the period 8 to 16 days posthatching; average initial weight was 82 g.

^b SAA, Sulfur amino acids; L-M, L-methionine; L-C, L-cystine; D and L isomers of homocysteine (HC) were provided as homocysteinethiolactone hydrochloride.

^c Isosulfurous to 0.25% L-cystine.

^d Isosulfurous to 0.30% L-methionine.

g/m mole. Using the slope-ratio technique, levels of intake up to 0.765 mmole resulted in 0.777 mmole of L-methionine activity/mmole of L-homocysteine (i.e., 28.30 g of gain/mmole of L-homocysteine \div 36.44 g of gain/mmole of L-methionine). Therefore, L-homocysteine, at intakes less than 0.765 mmole, was 77.7% as efficacious as L-methionine in supporting growth. Similarly, when intake exceeded 0.765 mmole the efficiency was reduced to 15.4% on a molar basis. This type of response is similar to that seen in nutrient requirement studies where no additional growth response occurs when intake of a nutrient is in excess of its physiological requirement for

growth. In this case, however, the response plateau would indicate a saturation of the transmethylation activity for reasons that can only be speculated. The two-slope response seen here may be the reason for conflicting data in the literature. Careful examination of the amount of homocysteine supplementation relative to this response curve is therefore important when evaluating the degree of homocysteine transmethylation.

Amos and co-workers (9) recently reported weight gains of 80 to 90% for rats fed homocysteine (provided as DL-homocysteinethiolactone · HCl) relative to rats fed DL-methionine. However, the basal

TABLE III. PERFORMANCE OF CHICKS FED GRADED LEVELS OF L-HOMOCYSTEINE AS A SOURCE OF METHIONINE (ASSAY 2)^{a,b}

Diet ^c	Source of SAA (%) ^d		Gain (g)	Gain/feed
	L-M	L-HC		
1	—	—	-3.5	-0.50
2	0.10	—	5.8	0.16
3	0.20	—	38.9	0.55
4	0.30	—	72.5	0.69
5	—	0.103	3.2	0.10
6	—	0.206	8.9	0.28
7	—	0.309	19.1	0.42
8	—	0.515	24.7	0.46
9	—	0.721	27.3	0.54
Pooled SE			2.3	0.04

^a Average of triplicate groups of five male chicks for the period 8 to 14 days posthatching; average initial weight was 64 g.

^b Diets 2 and 5, 3 and 6, and 4 and 7 are isosulfurous.

^c All diets contained 0.35% L-cystine.

^d L-Homocysteine (L-HC) was provided as L-homocysteinethiolactone hydrochloride; L-M, L-methionine.

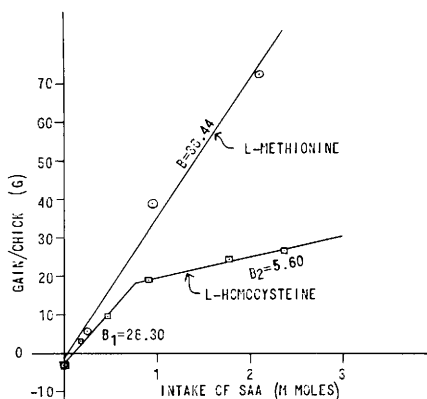


FIG. 2. Standard curve of gain per chick as a function of L-methionine intake and the broken-line response curve of L-homocysteine as a function of intake. B₁ and B₂ indicate slope values based on regression analysis of weight gain as a function of dietary intake.

diet of isolated soybean protein contained 0.2% methionine. Their 80-90% efficacy results were based on total gain of rats fed the basal diet supplemented with DL-homocysteine and DL-methionine. This approach actually compares the TSAA content of each diet and not the the gain due to DL-homocysteine per se. When weight gain due to homocysteine per se is plotted against actual intake, a value of 35% uti-

lization of homocysteine as a source of TSAA is the result. This is in agreement with our results showing that homocysteine is a poor source of methionine.

The plateau in growth at higher levels of homocysteine as seen in Fig. 2 may be due to a depletion of methyl donors for the transmethylation reaction. Klose and Almquist (7) reported poor gains in chicks fed DL-homocysteine in an arachin-based diet containing 0.20% supplemental choline chloride relative to those of chicks fed DL-methionine. When choline chloride was increased to 0.50%, weight gains were equal for both sources of SAA. The significance of this observation is hard to determine, however, because growth of the chicks was very poor. Chicks fed their basal diet which contained close to 0.5% SAA lost weight. Thus, some nutrient other than SAA must have been growth limiting.

Our basal diet contained 0.2% choline chloride, which is almost three times the growth requirement for chicks of this strain under our experimental conditions (12). It is therefore unlikely that choline was limiting in these assays. Results of assay 3 (Table IV) indicate that chicks fed L-homocysteine in the presence of 0.5% choline chloride showed no improvement in weight gain over chicks fed 0.2% choline chloride. Weight gains of chicks fed L-homocysteine at either level of choline chloride were inferior ($P < 0.01$) to those fed L-methionine.

TABLE IV. EFFECT OF EXCESS DIETARY CHOLINE ON THE EFFICACY OF L-HOMOCYSTEINE AS A SOURCE OF METHIONINE FOR THE YOUNG CHICK (ASSAY 3)^a

Diet ^b	Level of SAA (%) ^c		Choline-Cl (%)	Gain (g)	Gain/feed
	L-M	L-HC			
1	0.314	—	0.20	81.5	0.68
2	—	0.323	0.20	32.5	0.47
3	—	0.323	0.50	32.7	0.43
Pooled SE				2.8	0.03

^a Average of triplicate groups of five male chicks for the period 16 to 21 days posthatching; average initial weight was 99 g.

^b All diets contained 0.35% L-cystine.

^c L-M, L-methionine; L-HC, L-homocysteine provided as L-homocysteinethiolactone hydrochloride; all additions are isosulfurous.

TABLE V. PERFORMANCE OF CHICKS FED DL-HOMOCYSTEINE AS A SOURCE OF TOTAL SULFUR AMINO ACIDS (ASSAY 4)^a

Diet	Level of SAA (%) ^b			Gain (g)	Gain/feed
	L-M	L-C	DL-HC		
1	0.50	—	—	114.2	0.63
2	—	—	0.45	53.9	0.49
3	—	—	0.90	61.2	0.60
4	0.25	0.25	—	114.5	0.61
5	—	0.25	0.45	60.4	0.57
6	—	0.25	0.91	54.6	0.57
Pooled SE				4.4	0.02

^a Average of triplicate groups of seven male chicks for the period 7 to 17 days posthatching; average initial weight was 66 g.

^b L-M, L-methionine; L-C, L-cystine; DL-HC, DL-homocysteine.

Results of assay 4 (Table V) indicate that chicks fed DL-homocysteine as a source of SAA perform similarly to chicks fed DL-homocysteine as a source of methionine per se. Since it was established in assay 1 that homocysteine has a high cysteine-sparing value, the utilization of homocysteine as a source of SAA appears to be limited by its methionine activity. The results in these assays clearly indicate that the chick fed a crystalline amino acid diet cannot efficiently convert either D- or L-homocysteine to methionine via transmethylation. It would thus appear that either (a) some factor necessary to stimulate transmethylation activity is missing in a protein-free, L-amino acid diet or (b) avian species, in fact, cannot under any circumstances meet their entire requirement for dietary methionine via methylation of homocysteine.

Summary. Young male chicks were fed crystalline L-amino acid diets in assays designed to evaluate the capacity of D- and L-homocysteine to supply methionine and cysteine. L-Homocysteine had a cysteine-sparing value of 100% and a methionine-sparing value of 28% at the level fed. D-Homocysteine was utilized less efficiently than was L-homocysteine, having a cysteine-sparing value of 68% and a me-

thionine-sparing value of only 7%.

The methionine activity of L-homocysteine varied when evaluated in a cystine-adequate diet, being more efficiently utilized at low levels of intake. However, at every level fed, L-homocysteine was inferior to L-methionine in supporting growth. The presence of additional choline in the diet did not improve the performance of chicks fed L-homocysteine as a source of methionine. Performance of chicks fed DL-homocysteine as a source of total sulfur amino acids was equal to that of chicks fed DL-homocysteine as a source of methionine per se. The utilization of the D and L isomers of homocysteine appears to be limited by the methionine activity they can replace.

The results of these assays clearly indicate that young chicks fed a crystalline L-amino acid diet cannot efficiently convert either D- or L-homocysteine to methionine via transmethylation.

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